

Letter to the Editor

A CRITIQUE ON COMMENTARY 'HOW *MYCOBACTERIUM LEPRAE* INFECTS PERIPHERAL NERVES' BY FREEDMAN *ET AL.*

Sir

Understanding of the mechanism/s of entry of *Mycobacterium leprae* into the peripheral nerve compartment and damage to neural constituents is extremely important in its prevention or modulation. In this context, the recent findings of Rambukkana and co-workers are indeed very important. We read with interest a science commentary 'How *Mycobacterium leprae* infects peripheral nerves' by Freedman and co-workers; that appeared in the June 1999 issue of *Leprosy Review* (70: 136–139) and another comment by the same group.¹ The authors postulate the mechanism of nerve infection in leprosy on the basis of recent findings by Rambukkana and coworkers^{2,3} which calls for certain clarifications.

The first paper of Rambukkana and colleagues² suggested the 'G' domain of the laminin $\alpha 2$ chain as a host-derived bridging molecule, and proposed that *M. leprae* interacts with host Schwann cells by binding to the $\alpha 6\beta 4$ integrin which acts as a laminin receptor on the Schwann cell surface. These primary Schwann cells and Schwann cell neuron co-cultures were devoid of the fibronectin that is normally present in their basal lamina *in vivo*. In their second paper,³ α -dystroglycan is proposed as a Schwann cell receptor for the *M. leprae*–laminin complex and these primary rat Schwann cell cultures lacked the integrin $\beta 4$ subunit. Authors suggest that the presence of both $\alpha 2$ -laminin and α -dystroglycan is responsible for restricting leprosy infection to the peripheral nervous system as these molecules are absent in the central nervous system.

Adherence of *M. leprae* to host cells has been well studied. Complement-mediated adherence to macrophages is known to occur via mannose receptors and not via FC receptor.⁴ The work of Lad and Mahadevan⁵ suggests involvement of the carbohydrate moiety. There are indications that adherence of *M. leprae* to Schwann cells is mediated through a lipid/polysaccharide moiety, i.e. antigens such as LAM and PGL 6,7 and to a 25–28 kDa glycoprotein obtained from peripheral nerve homogenates of humans and rats.⁸ The fibronectin, an ECM protein, and a component of basal lamina, is also shown to be involved in *M. leprae* binding to Schwann cells.⁹ Therefore a multitude of factors have been shown to play a role.

Further, in their hypothesis, Freedman and coworkers describe bacillation of 'myelinated fibre' Schwann cells which in turn leads to demyelination and associated sensory impairment observed in leprosy. *In vitro* studies by Rambukkana and coworkers,² i.e. binding assays using immobilized ECM proteins, primary rat Schwann cell cultures, Schwann cell neuron co-cultures and using cryosections of wild type and dystrophic *dy/dy* mice forms the basis of this hypothesis. In the second paper, Rambukkana and co-workers³ have also used immortalized human Schwann cells; these, however, showed decreased binding to *M. leprae* as compared to primary rat Schwann cells.

We believe that the following points have direct relevance to the molecular mechanisms of *M. leprae* infection and nerve damage in leprosy. First and foremost, it has been well established that in human leprosy *M. leprae* are rarely seen in the Schwann cells of the myelinated fibres (only

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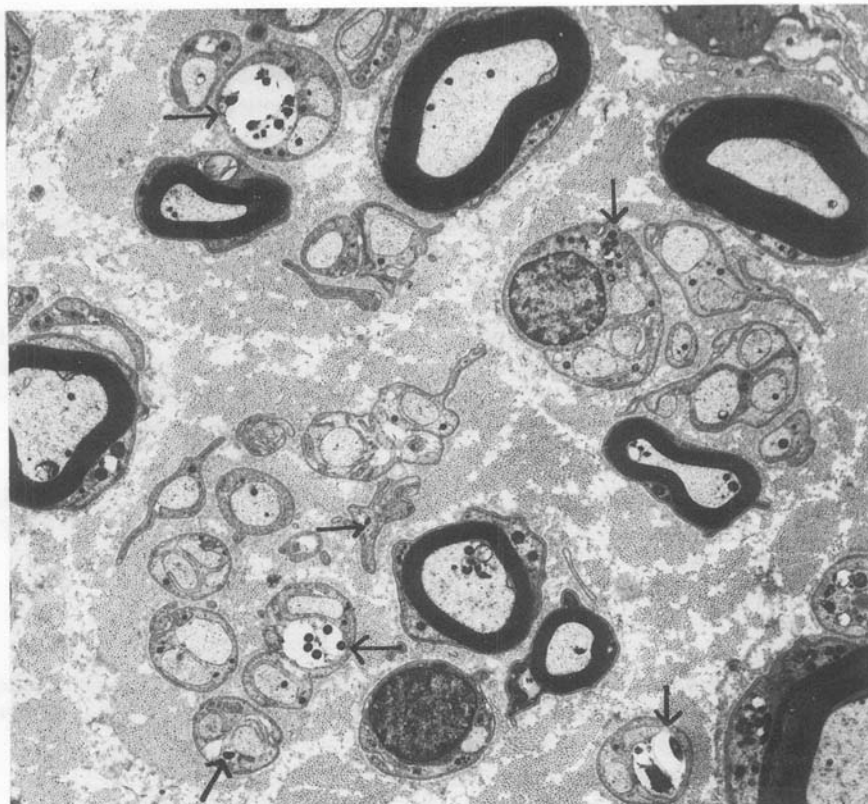


Figure 1. Sural nerve biopsy obtained from an untreated lepromatous leprosy case at an early stage of infection. Note the presence of large globi of solidly stained bacilli in six of the nonmyelinated fibre schwann cell units (arrow) (magnification $\times 3500$).

4–5% in advanced lepromatous leprosy). The Schwann cells of the non-myelinated ‘C’ fibres are the ones to harbour bacilli (70–80%) (see Figure 1) in the early stage^{10,11} This implies that there is no direct correlation between demyelination and infection with *M. leprae* in the Schwann cells. Secondly, and more importantly, this difference also needs to be explained in extrapolating the results of Rambukkana and co-workers that was using culture system.

Since the lamina forms the component of the basal lamina in both non-myelinated and myelinated fibre Schwann cells; and if the spread of *M. leprae* into the nerve is via the circulation as has been proposed by Freedman *et al.*, the preferential bacillation of the non-myelinated fibre Schwann cells remain unexplained.

Secondly, it is also important to note that in almost all the animal models studied so far *M. leprae* rarely enters the peripheral nerves and in particular the Schwann cells; this is not so in humans. The mouse foot pad model for leprosy is extensively investigated from the point of view of understanding peripheral neuropathy associated with leprosy. Reproducible pathological changes in the sciatic nerves following foot pad inoculation with *M. leprae* are well documented.^{12,13} However, *M. leprae* and inflammatory cells are conspicuously absent in these lesions.¹³ In armadillos, despite disseminated infection with *M. leprae*,¹⁴ the nerve and the Schwann cells in particular seldom show invasion of *M. leprae*. In an attempt to infect Schwann cells *in vivo* through intraneural inoculation with viable *M. leprae*,^{15,16} xenografting of leprosy nerve into immunosuppressed mice¹⁷ has been undertaken.

It was concluded that Schwann cells in mice are highly resistant to *M. leprae* infection, regardless of route of infection.

Following foot pad inoculation in mice, *M. leprae* colonizes the striated muscle fibres.¹⁸ In armadillos, predominance of endothelial cell bacillation has been reported.¹⁹ In our view, this difference could be a reflection of a species-specific difference in tissue tropism.

Qualitative and quantitative morphological studies of leprosy nerves, on the other hand, have shown that atrophic changes in the axonal compartment precede demyelination, indicating the axon to be the primary site of damage.^{11,20,21} In an independent study, Shetty and coworkers²² report detection of multitude of *M. leprae* antigens (including LAM and PGL) in the axonal compartment of both tuberculoid and lepromatous leprosy nerves. In a recent study by us (unpublished data) using SDS-PAGE and WB analysis, it was noted that inoculation into the hind foot pads of S/W mice, with both viable and heat killed *M. leprae*, brings about loss of immunoreactivity to major cytoskeletal proteins of sciatic nerves, again supporting the role of antigens. The model suggested by Freedman and co-workers may have some relevance in this regard, and *M. leprae* antigens such as LAM/PGL and not integral *M. leprae* binding to myelinated fibre Schwann cells may lead to disruption of the cytoskeletal proteins leading to demyelination.

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We are publishing this letter with sincere apologies to the authors. The letter was submitted in reply to the article by Freedman et al. 1999 (How leprosy affects peripheral nerves. *Lepr Rev* 70: 136–139.) The letter was accepted in August 1999 but was then lost.

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